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1,2-DIOXETANE DERIVATIVES, LUMINESCENT REAGENTS,
LUMINESCENCE METHODS AND MEASURING METHODS

20444-0000-2445001
The present invention relates to 1,2-dioxetane derivatives. The 1,2-dioxetane derivatives of the present invention are compounds which are capable of inducing chemiluminescence and can be used, for example, as substrates for immunoassay.

Heretofore, various 1,2-dioxetane derivatives have been synthesized, and it is known that compounds having a spiroadamantyl group bonded at the 3-position, are useful as chemiluminescent substrates (see, for example, JP-B-5-21918, and JP-B-5-45590). Further, as produced by the present inventors, compounds disclosed in JP-A-8-245615, JP-A-8-169885 and JP-A-8-165287, are known. However, these 1,2-dioxetane derivatives were poor in thermal stability. JP-A-9-216887 discloses compounds having the thermal stability improved.

As mentioned above, various studies have been made with respect to 1,2-dioxetane derivatives, and various compounds have been produced. However, for such

compounds to be useful in the field of e.g. clinical tests, the compounds themselves are required to be stable and easy to handle and capable of emitting light at high efficiency. Accordingly, it has been desired to develop
5 a compound superior to conventional compounds.

Conventional compounds including the compounds disclosed in the above-mentioned JP-A-9-216887, were poor in luminous efficiency in a protic solvent of e.g. an aqueous type, and even if they were employed for an
10 immunoassay in a practical clinical test, they were unable to provide practically sufficient strength, if a protic solvent is used as a measuring condition. Accordingly, at the time of measurement, a substance which enhances luminescence, other than the 1,2-dioxetane
15 derivatives, was required to be present as an enhancer.

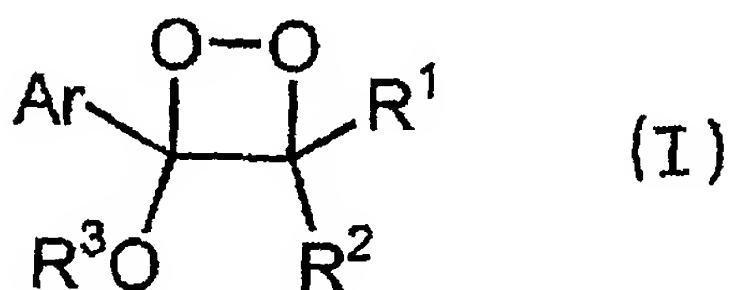
As enhancers, cationic surfactants (such as cetyltrimethylammonium bromide and cetyldimethylbenzylammonium chloride), water-soluble polymerized quaternary onium salts, (including quaternary
20 ammonium salts, quaternary sulfonium salts and quaternary phosphonium salts, such as poly(vinylbenzyltrimethylbenzylammonium chloride), poly(vinylbenzyltrimethylammonium chloride) and poly(vinylbenzyltributylammonium chloride)), natural
25 polymers (such as serum albumin, immunoglobulin and serum lipoprotein of mammals), etc., were used. However, when these enhancers were used, the viscosity tended to be

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high, whereby a due care was required in handling.
Accordingly, if there is a compound which is capable of
showing high luminous efficiency without using any
enhancer even in a protic solvent, such a compound is
5 more useful.

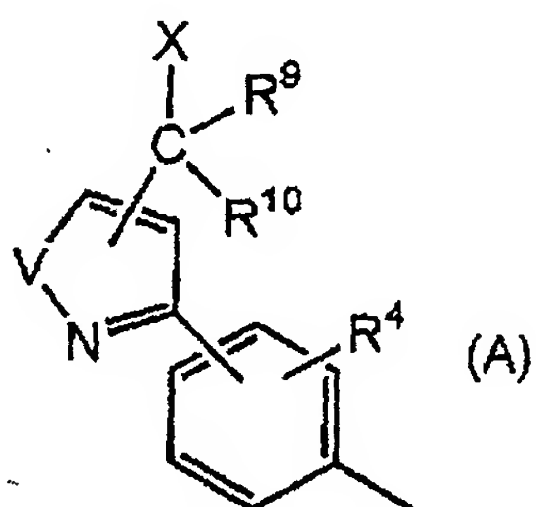
Under these circumstances, the present inventors
have conducted an extensive study to develop a compound
which is superior to conventional compounds and as a
result, have found that a 1,2-dioxetane derivative having
10 an aromatic ring substituent having a 5-membered hetero
ring such as an isoxazole ring of the following formula
(A) or (B) bonded thereto, exhibits high luminous
efficiency even without using any enhancer even in a
protic solvent such as water. The present invention has
15 been accomplished on the basis of this discovery.

Namely, the present invention provides a 1,2-
dioxetane derivative of the formula (I):

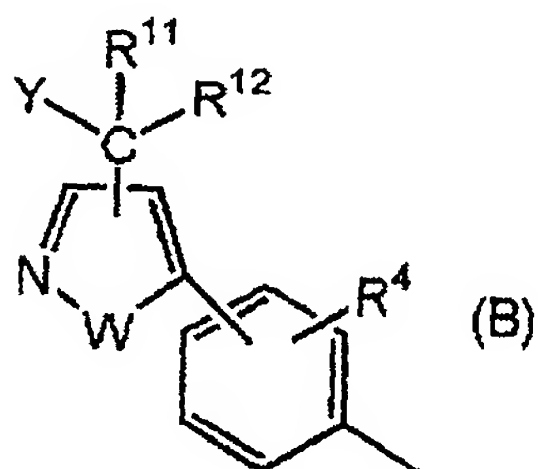


wherein each of R¹ and R² which are independent of each
20 other, is a hydrogen atom, an alkyl group or an aryl
group, or R¹ and R² may together form a cyclic or
polycyclic organic ring group spiro-bonded to the
dioxetane ring, R³ is an alkyl group or an aryl group, or

R^3 and R^1 or R^2 may together form a condensed ring containing the dioxetane ring and a hetero atom, and Ar is a group of the formula (A):



- 5 wherein R^4 is a hydroxyl group, an alkoxyl group, an aralkyloxy group, a group of $-\text{OSi}(R^5R^6R^7)$ (wherein each of R^5 , R^6 and R^7 which are independent of one another, is an alkyl group or an aryl group), a phosphate group or a group of $-\text{S}(\text{C}=\text{O})R^8$ (wherein R^8 is an alkyl group or an
- 10 aryl group), each of R^9 and R^{10} which are independent of each other, is a hydrogen atom, an alkyl group, an aryl group or a halogen atom, X is a halogen atom, and V is an oxygen atom or a sulfur atom, or a group of the formula (B):



15

wherein R^4 is the same as R^4 in the above formula (A), each of R^{11} and R^{12} which are independent of each other,

is a hydrogen atom, an alkyl group, an aryl group or a halogen atom, Y is a halogen atom, and W is an oxygen atom or a sulfur atom.

Further, the present invention provides a
5 chemiluminescent reagent which contains the above 1,2-dioxetane derivative. Further, the present invention provides a luminescence method which comprises decomposing the above 1,2-dioxetane derivative to have chemiluminescence generated. Still further, the present
10 invention provides a measuring method which comprises measuring a substance to be detected, in a test sample, by means of the above luminescence method. Furthermore, the present invention provides a luminescence method which comprises letting a compound having a 1,2-dioxetane
15 structure emit light in a protic solvent in the absence of any other enhancer.

Now, the present invention will be described in further detail with reference to the preferred embodiments.

20 In this specification, "an alkyl group" means a C₁₋₂₀ straight chain, branched or cyclic alkyl group which may have a substituent, and the alkyl group is a straight chain group such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl,
25 tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl or icosanyl, a group in which such an alkyl group is branched, or a group in which such an alkyl

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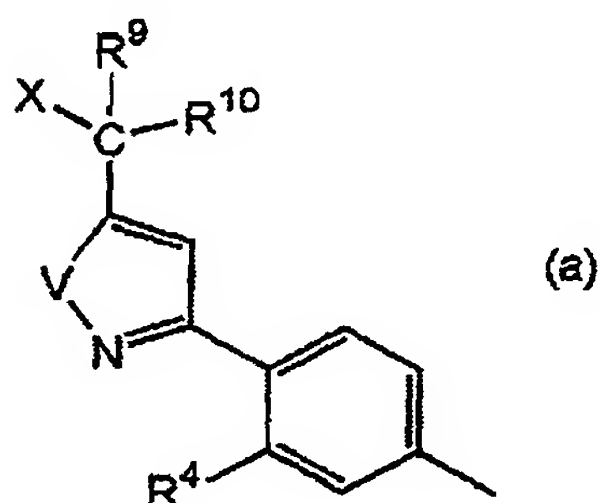
group is cyclic. The substituent which such an alkyl group may have, is, for example, a hydroxyl group, an alkoxyl group or an aryl group. The alkoxyl group may, for example, be one having from 1 to 5 C₁₋₂₀ alkoxyl groups bonded in a straight chain form or in a branched form, such as methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, methoxyethoxy, methoxypropoxy, ethoxyethoxy, ethoxypropoxy or methoxyethoxyethoxy. Further, the above aryl group may, for example, be a C₆₋₂₀ aromatic hydrocarbon group such as phenyl or naphthyl, or a heteroaryl group having from 1 to 5 nitrogen atoms, oxygen atoms or sulfur atoms in a ring, such as furyl, thienyl or pyridyl.

Further, in this specification, "an alkoxyl group" may be the same as the alkoxyl group which may be substituted on the above alkyl group, and "an aryl group" may be the same as the aryl group which may be substituted on the above alkyl group. Further, in this specification, "a polycyclic organic ring group" is a C₆₋₃₀ polycyclic alkylene which may optionally be substituted from 1 to 10 groups independently selected from C₁₋₁₀ alkyl, C₁₋₁₀ alkoxyl, halogen and halo-C₁₋₁₀ alkyl, such as an adamantyl group or a bicyclo[2.2.1]heptyl group. Further, a halogen atom, an alkyl group, an aryl group, a cyano group, an amide group, an alkoxyl group, or a carboxyl group may be bonded to optional carbon of the polycyclic organic ring group. Further, "an

aralkyloxy group" is a C₇₋₂₀ group such as benzyloxy or phenethyloxy, and "a halogen atom" may, for example, be fluorine, chlorine or bromine.

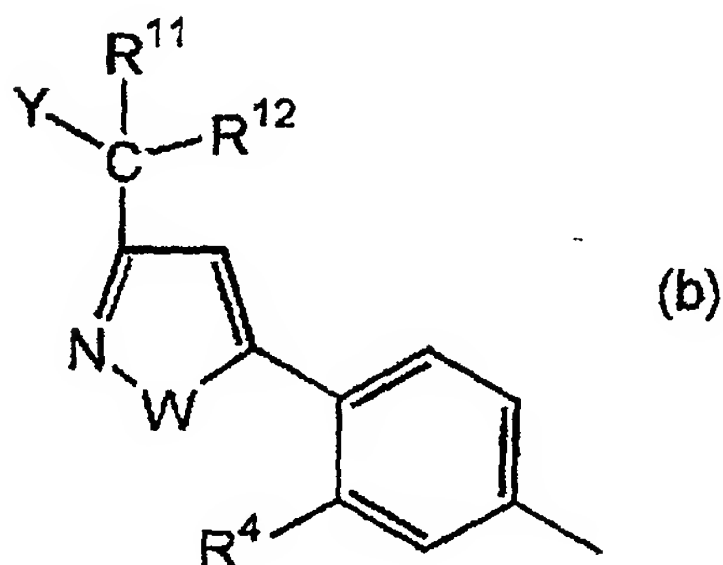
Further, the case wherein in the formula (I), R³ and R¹ or R² together form a condensed ring containing the dioxetane ring and a hetero atom, may, for example, be a condensed ring of the dioxetane ring and a furan ring, or a condensed ring of the dioxetane ring and a pyran ring.

In the present invention, preferred is one wherein in the above formula (I), wherein Ar is a group of the formula (a):



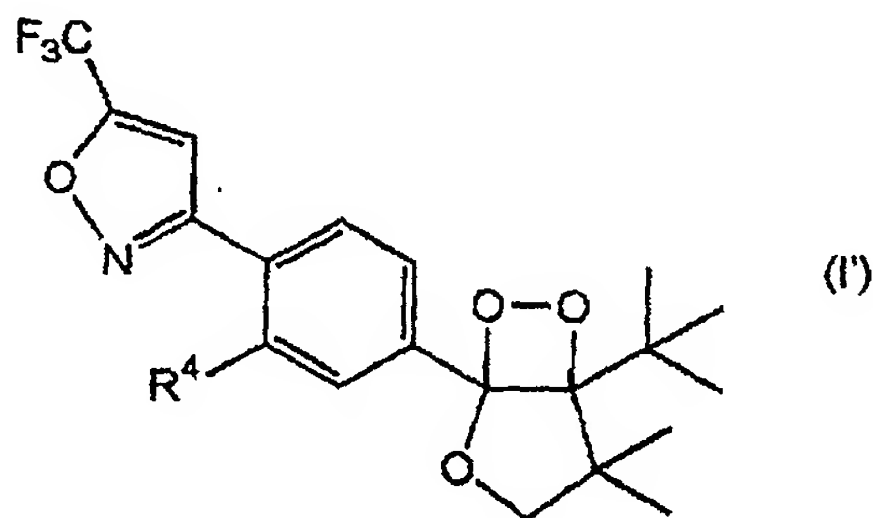
wherein R⁴, R⁹, R¹⁰, X and V are the same as R⁴, R⁹, R¹⁰, X and V in the above formula (A), or a group of the formula

(b):



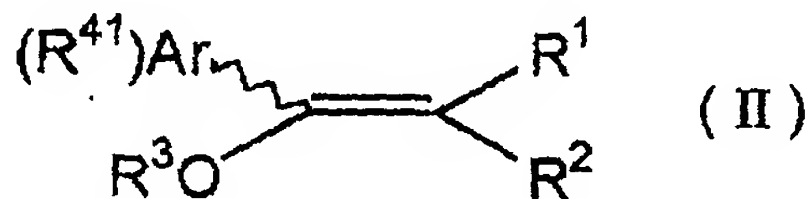
wherein R^4 , R^{11} , R^{12} , Y and W are the same as R^4 , R^{11} , R^{12} , Y and W in the above formula (B).

It is preferred that in the formula (I), R^3 and R^1 or R^2 together form a condensed ring of a dioxetane ring and a furan ring, and more preferably, R^2 or R^1 which does not form the condensed ring together with R^3 , is a C_{3-4} alkyl group. Particularly preferred is a compound represented by the formula (I'):



wherein R^4 is as defined above.

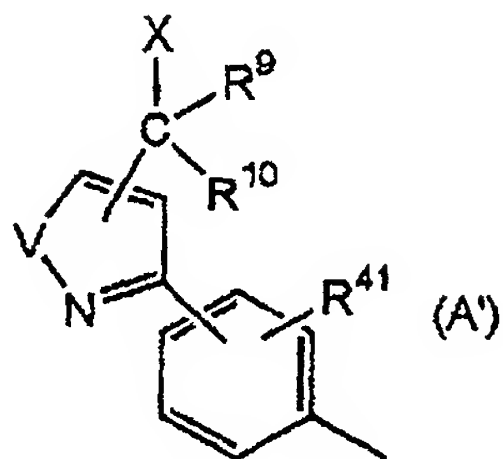
The 1,2-dioxetane derivative of the formula (I) of the present invention can be produced in accordance with the following reaction scheme from an enol ether derivative of the formula (II):



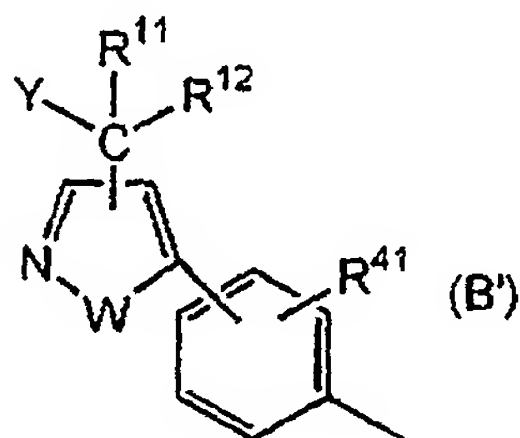
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wherein R^1 to R^3 are the same as R^1 to R^3 in the formula (I), R^{41} is an alkoxyl group or an aralkyloxy group, and $(R^{41})Ar$ is an aryl group substituted by R^{41} , represented

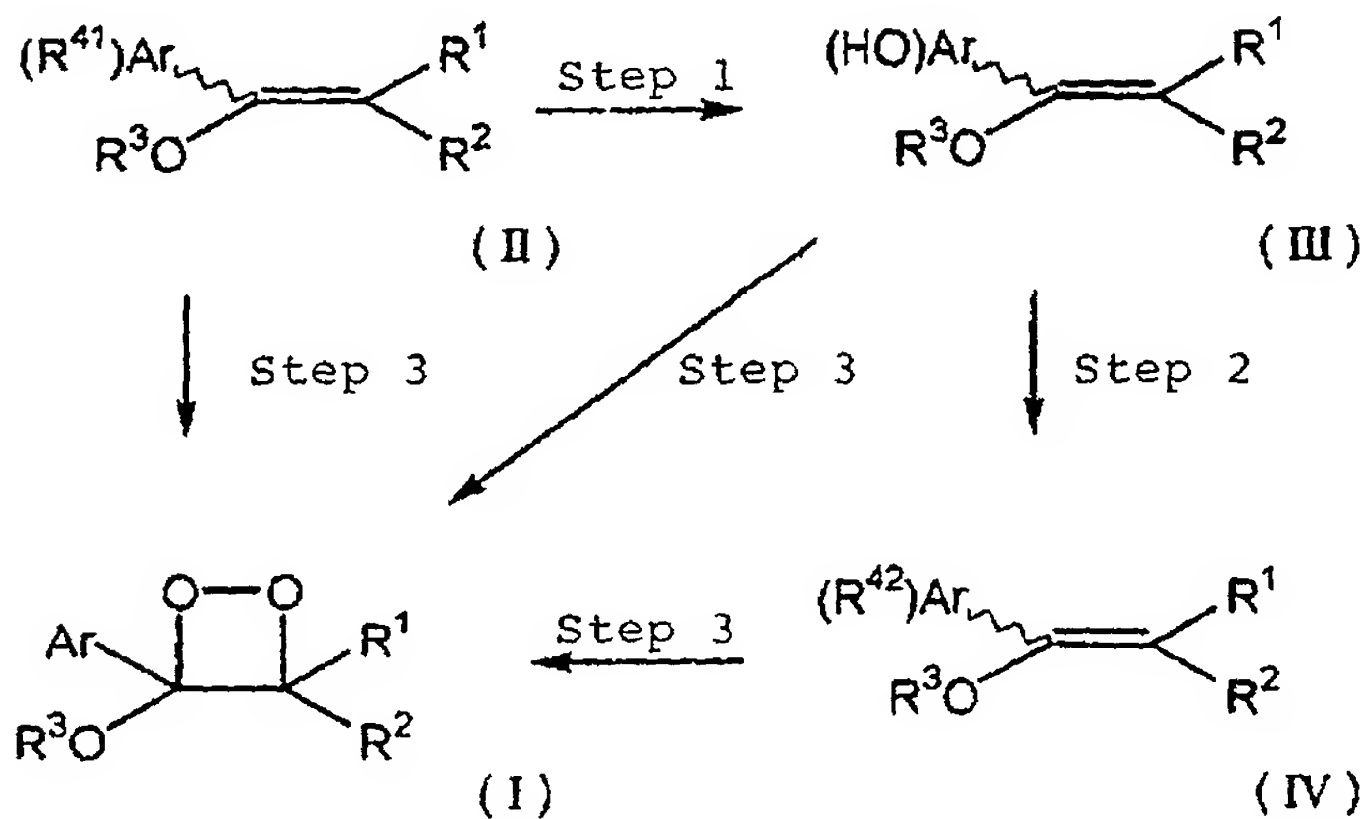
by a group of the formula (A'):



wherein R⁹, R¹⁰, X and V are the same as R⁹, R¹⁰, X and V in the above formula (A), and R⁴¹ is the same as R⁴¹ in the above formula (II) or a group of the formula (B'):



wherein R¹¹, R¹², Y and W are the same as R¹¹, R¹², Y and W in the above formula (B), and R⁴¹ is the same as R⁴¹ in the above formula (II).



$$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^{13}\text{O}-\text{P}-\text{O}- \\ \diagup \\ \text{R}^{14}\text{O} \end{array}$$

(wherein each of R^{13} and R^{14} is an alkali metal, a quaternary ammonium salt or an alkyl group, or R^{13} and R^{14} may together form a ring). The group of (HO)Ar in the compound of the formula (III) is one having an OH group at the same position as the position of substituent R^{41} in the formula (II), and $(R^{42})Ar$ in the formula (IV) is one having a substituent R^{42} at the same position as the position of the substituent R^{41} in the formula (II).

Step 1: In this step, a compound of the formula (II) is subjected to a protective group-removing reaction to produce a compound of the formula (III). The compound which is subjected to the protective group-removing reaction is a compound of the above formula (II), wherein R^1 to R^3 are as defined above, and R^{41} is a protective group for a hydroxyl group (preferably a methoxy group or a benzyloxy group). Such a reaction can be carried out by a method well known to those skilled in the art, i.e. by reacting it with an anion of an alkylthiol, or by subjecting it to a hydrogenation reaction. Either

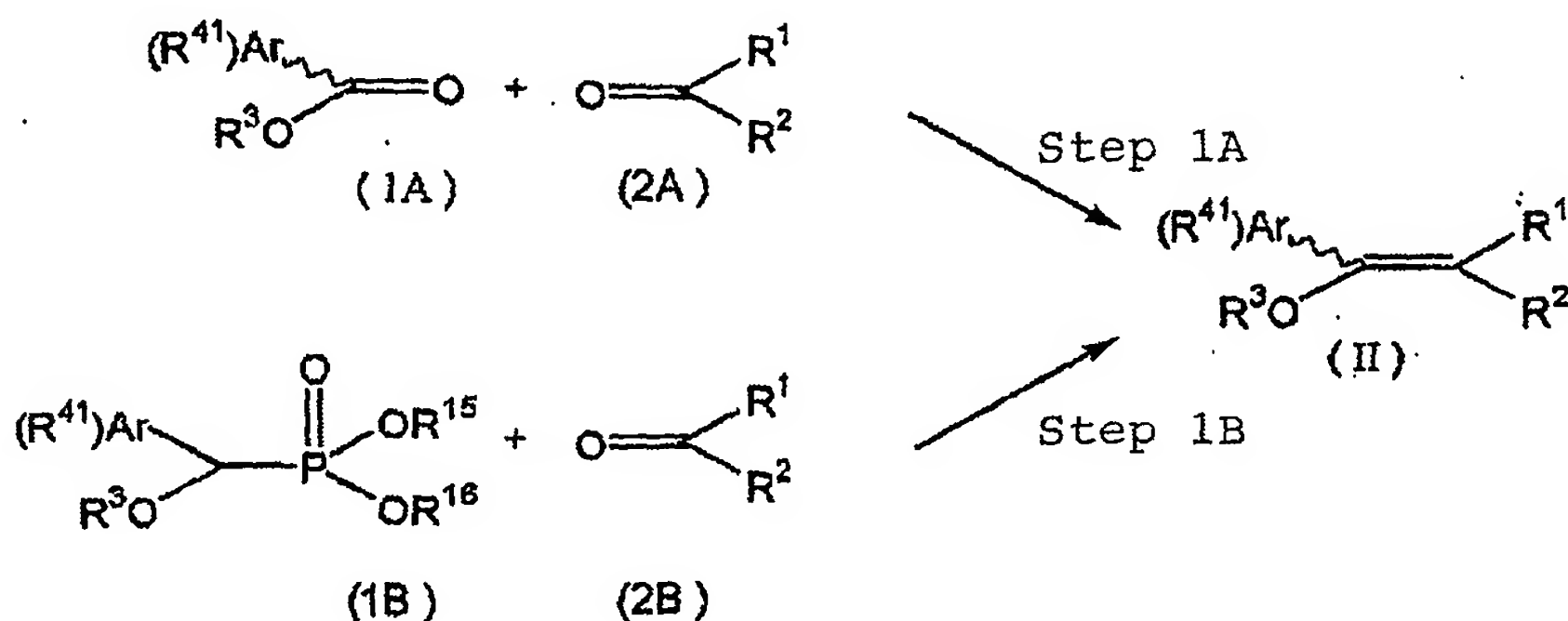
reaction may be selected for use depending upon the group to be removed.

Step 2: In this step, in order to form a silyloxy group or a phosphoric acid group, the corresponding halogenated trialcoxysilane or halogenated phosphate is reacted to the compound of the above formula (III) to produce a compound of the formula (IV). In this step, for example, if chloroethylene phosphate is reacted in order to introduce a phosphoric acid group, the product can be converted by sodium cyanide to a sodium salt of cyanoethyl phosphate, and the cyanoethyl group is further removed, followed by conversion to an ammonium sodium salt. This ammonium sodium salt can easily be converted to a disodium salt, for example, by a reaction with sodium hydrogencarbonate.

Step 3: In this step, the compound of the formula (II), (III) or (IV) is reacted with singlet oxygen to produce a 1,2-dioxetane derivative of the formula (I). The reaction with singlet oxygen can be accomplished by subjecting the enol ether derivative of the above formula (II), (III) or (IV) to visible light irradiation in an oxygen atmosphere in the co-existence of a photosensitizer such as Methylene Blue, Rose Bengale or tetraphenylporphine (TPP). Here, as a solvent, a halogenated hydrocarbon such as dichloromethane, dichloroethane or carbon tetrachloride, or an alcohol such as methanol or ethanol, may be employed. Further,

the reaction is preferably carried out at a temperature of from -80°C to room temperature.

The following method may, for example, be mentioned as a method for producing the enol ether derivative of the above formula (II).



In the above formulae, R^1 to R^3 and R^{41} are the same as R^1 to R^3 and R^{41} in the above formulae (I) and (II). Each of R^{15} and R^{16} is an alkyl group, or R^{15} and R^{16} may together form a ring.

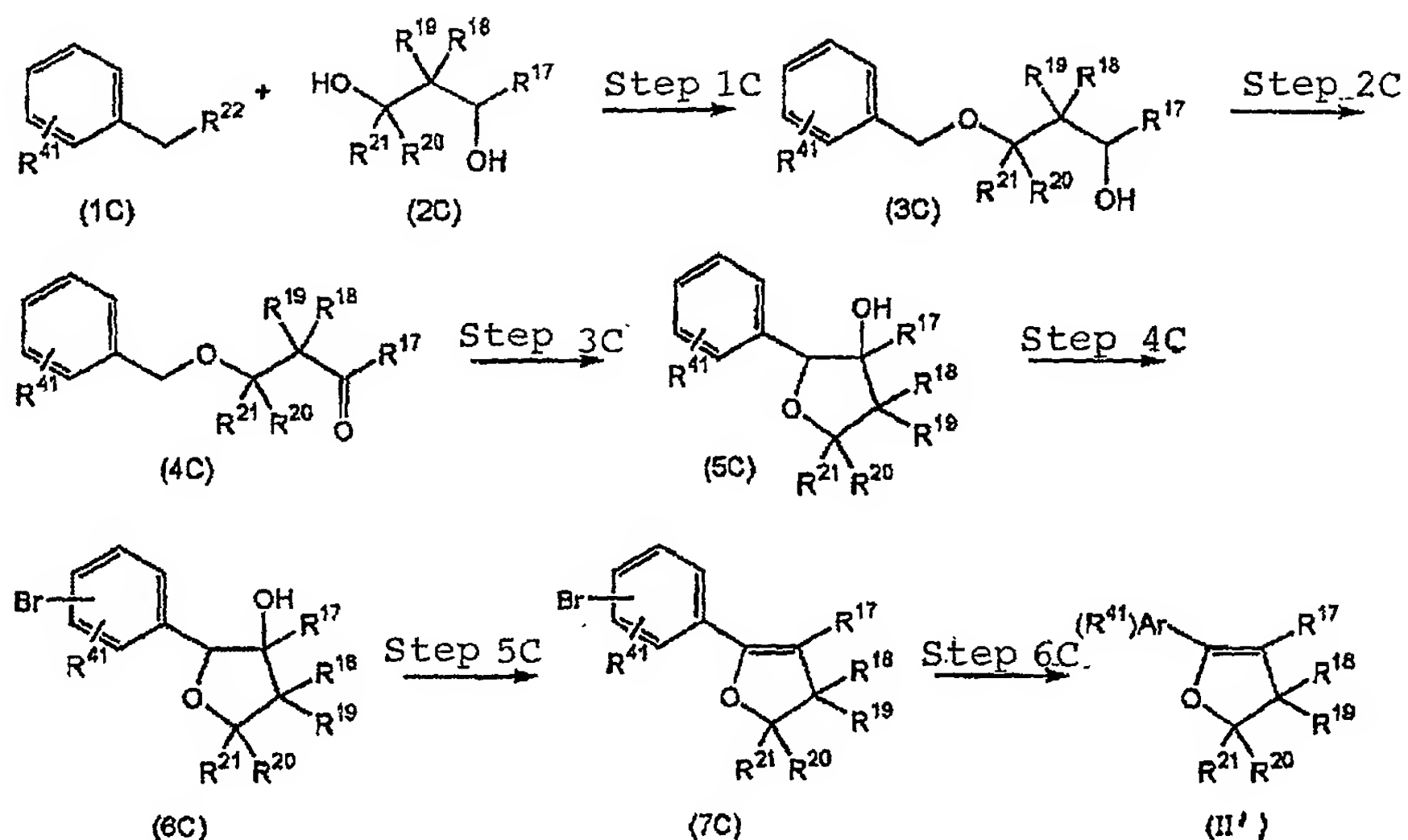
Step 1A: In this step, an aromatic carboxylic acid ester of the formula (1A) is reacted with a ketone of the formula (2A) to produce an enol ether derivative of the formula (II). The reaction is carried out in the presence of titanium, as an essential requirement. It is usually preferred that titanium is formed into a reduced state by treating titanium halide such as titanium chloride with a reducing agent such as lithium aluminum hydride and a base such as triethylamine, and then used for the reaction. The reaction may be carried out in an organic ether such as tetrahydrofuran (THF). The

reaction may proceed at a temperature of from 0 to 100°C, but the reaction is preferably carried out under reflux of THF, from the viewpoint of the operation efficiency and reactivity.

- 5 Step 1B: In this step, an arylmethylphosphonate of the formula (1B) is reacted with a ketone of the above formula (2B) to produce an enol ether derivative of the above formula (II).

20 The arylmethylphosphonate of the above formula (1B) is a compound which can easily be produced by the method disclosed in the above-mentioned JP-B-5-45590. It is preferred that diisopropylamine is treated with butyl lithium or the like to form a lithium amide, which is used for the reaction. The reaction can be carried out
15 in an organic ether such as tetrahydrofuran (THF). The reaction is preferably carried out at a temperature of from -78°C to room temperature.

In a case where the compound of the above formula (II) is a dihydrofuran derivative, the following method
20 may, for example, be mentioned as the method for its production.



In the above formulae, each of R^{17} , R^{18} , R^{19} , R^{20} and R^{21} which are independent of one another, is a hydrogen atom, an alkyl group or an aryl group. Further, each pair of R^{18} and R^{19} , R^{20} and R^{21} , R^{17} and R^{19} , R^{17} and R^{20} , and R^{18} and R^{20} , which are independent of one another, may form a cyclic alkyl group. R^{41} is as defined above. R^{22} is a halogen atom, a substituted sulfonyloxy group or a hydroxyl group.

Step 1C: In this step, a compound of the above formula (1C) is reacted with a compound of the above formula (2C) to produce a compound of the above formula (3C). The reaction can be accomplished by a so-called Williamson synthesis. Here, in a case where substituent R^{22} of the compound of the formula (1C) is a halogen atom or a substituted sulfonyloxy group, such a compound can be subjected directly to the reaction, and in a case where

R²² is a hydroxyl group, such a group is converted to a sulfonyloxy group by e.g. tosyl halide in the reaction system, and then the compound is subjected to the reaction, to accomplish this step.

5 Step 2C: In this step, the compound of the above formula (3C) is oxidized to produce a compound of the above formula (4C). The oxidation in this step can be carried out by means of a chromium type oxidizing agent or an activating agent. As the chromium type oxidizing agent,
10 pyridinium chlorochromate (PCC) or pyridinium dichlorochromate (PDC) may, for example, be used. At that time, a halogenated hydrocarbon such as dichloromethane may be used as the solvent. Further, in a case where the above-mentioned activating agent is
15 employed, the reaction can be carried out by a combination with a solvent, such as a Py· SO₃/triethylamine/DMSO, Ac₂O/DMSO system.

Step 3C: In this step, the compound of the above formula (4C) is subjected to ring closure to produce a compound
20 of the above formula (5C). The reaction is carried out by using a lithium salt of a secondary amine such as lithium diisopropylamide, or a base such as t-butoxypotassium. As the solvent, an organic solvent such as THF or DMSO, may be employed. The reaction is
25 preferably carried out at a temperature of from 0°C to room temperature for from 1 to 5 hours.

Step 4C: In this step, the compound of the above formula

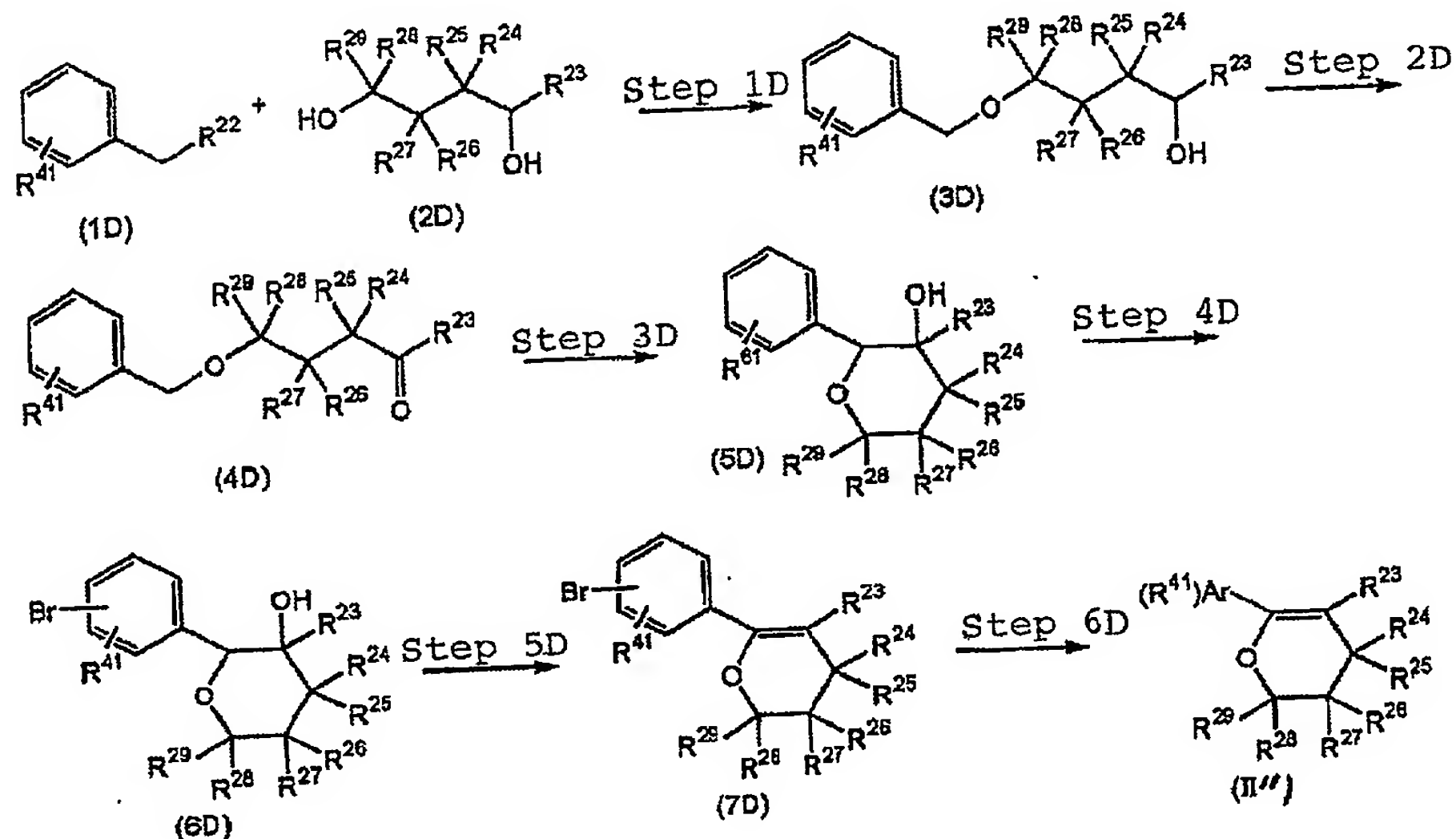
(5C) is brominated to produce a compound of the above formula (6C). The reaction is carried out by using a brominating agent such as N-bromosuccinimide. As the solvent, an organic solvent such as water-containing THF, dioxane or DMF, may be used.

Step 5C: In this step, the compound of the above formula (6C) is dehydrated to produce a compound of the above formula (7C). The reaction is carried out by reacting thionyl chloride in the presence of a base such as pyridine, or by using as a catalyst an acid such as phosphoric acid or p-toluenesulfonic acid. As the solvent, a halogenated hydrocarbon such as methylene chloride, or an aromatic hydrocarbon such as toluene, may be employed, and the solvent may suitably be selected for use depending upon the reagent to be reacted.

Step 6C: In this step, from the compound of the above formula (7C), a compound of the above formula (II') is produced. The reaction is such that the bromine of the compound of the above formula (7C) is substituted to introduce the desired substituent to produce the compound of the above formula (II'). A substituted amino group may be introduced in such a manner that a carboxyl group is introduced by means of a lithium salt such as butyl lithium, followed by reaction with an amine or ammonia by using carbonylimidazole as a condensing agent. Further, from the amide produced by the above reaction, for example, a compound having an oxazoline ring may be

obtained by reacting substituted or unsubstituted
ethanolamine. Further, an acyl group may be introduced
in such a manner that a lithium salt such as butyl
lithium is employed in the same manner as above and is
5 reacted with N-methylformanilide or with an aldehyde such
as acetaldehyde or benzaldehyde, followed by oxidizing
the hydroxyl β group by an oxidizing agent such as
manganese dioxide. Here, the compound having an acyl
group is subjected to conversion of the acyl group to a
10 hydroxyimino group, and such a dianion is reacted with a
corresponding amide or ester, followed by dehydration to
form an isoxazole ring. As an another method, the
compound having an acyl group introduced is subjected to
conversion to a β -diketone type substituent, and a
15 hydroxylamine is reacted thereto to form an isoxazole
ring.

In a case where the compound of the above formula
(II) is a dihydropyran derivative, the following method,
may, for example, be mentioned as the method for its
20 production.



In the above formulae, each of R²³, R²⁴, R²⁵, R²⁶, R²⁷, R²⁸ and R²⁹ which are independent of one another, is a hydrogen atom, an alkyl group or an aryl group. Further, each pair of R²⁴ and R²⁵, R²⁶ and R²⁷, R²⁸ and R²⁹, R²³ and R²⁴, R²³ and R²⁶, R²³ and R²⁸, R²⁴ and R²⁶, R²⁴ and R²⁸, and R²⁶ and R²⁸, which are independent of one another, may together form a cyclic alkyl group. R⁴¹ and R²² are as defined above.

Steps 1D, 2D, 3D, 4D, 5D and 6D: The process for producing the compound of the above formula (7D) can be accomplished in the same manner as the above Steps 1C, 2C, 3C, 4C, 5C and 6C.

The 1,2-dioxetane derivative of the formula (I) of the present invention is decomposed into a carbonyl compound in an alkaline condition accompanying chemiluminescence, and it will be decomposed also by an

esterase (a carboxylate hydrolase) such as an aryl
esterase or acetylcholine esterase, an enzyme such as an
alkaline phosphatase, a fluoro compound such as
tetrabutylammonium fluoride, or an acidic or amine
5 compound, accompanying chemiluminescence.

Accordingly, the 1,2-dioxetane derivative of the
formula (I) can be a chemiluminescent reagent. The
decomposition of the 1,2-dioxetane derivative
accompanying such chemiluminescence, may be carried out
10 in the presence of other enhancer, or may be carried out
in the absence of any other enhancer. It is one of
characteristics that the 1,2-dioxetane derivative of the
formula (I) exhibits high luminous quantum yield even if
the decomposition accompanying chemiluminescence is
15 carried out in a protic solvent and in the absence of any
other enhancer. The luminous quantum yield is preferably
at least 1%, more preferably at least 10%, particularly
preferably at least 20%.

Further, the chemiluminescent reagent of the present
20 invention can be used for all measuring methods intended
to obtain the concentrations of substances to be detected
in test samples. For example, it can be used as a
reagent for measuring immunity in an immunoassay, and
further, it can be used also in an enzyme detecting
25 method, a chemical detecting method, a nucleotide probe
method.

Substances to be detected in the above immunoassay

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include, for example, hormones such as hCG, TSH and LH, cancer-related substances such as AFP and CEA, viral antigens and antibodies such as HIV and HTLV-I, and nucleic acids (DNA, RNA).

5 The above immunoassay can be carried out by a step of preliminarily bonding the above enzyme to a substance having a specific affinity to the substance to be detected as mentioned above, and mixing it with a test sample containing the substance to be detected, reacting
10 the mixture for a predetermined period of time and bonding the substance to be detected in the test sample to the substance having the affinity thereto, and a step of determining the amount of the substance having the affinity, bonded or not bonded. The above step of
15 determining the amount of the substance having the affinity, bonded or not bonded, is carried out in such a manner that the enzyme and the 1,2-dioxetane derivative of the present invention are reacted, whereby the luminescence intensity from the 1,2-dioxetane derivative
20 increases in proportion to the amount of the enzyme, whereby the concentration of the substance can be obtained by measuring the luminescence intensity.

 The reagent for immunoassay containing the 1,2-dioxetane derivative of the present invention, and the
25 above-mentioned immunoassay employing it, are also included in the present invention.

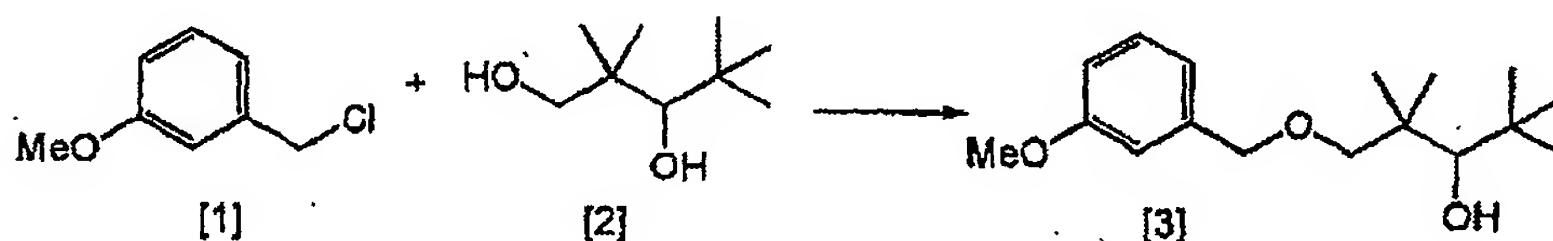
 The 1,2-dioxetane derivative of the formula (I) of

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the present invention is capable of exhibiting stable
luminous efficiency with high quantum yield and is a
stable compound having high thermal stability, whereby
depending upon the cold storage, it is stable to such an
5 extent that no decomposition product is observed upon
expiration of one year. Accordingly, measurement of
luminescence can be carried out simply and efficiently,
and it is useful, for example, in the field of clinical
tests.

10 Now, the present invention will be described in
further detail with reference to Examples and Reference
Examples. However, it should be understood that the
present invention is by no means restricted to such
Examples.

15 REFERENCE EXAMPLE 1



In a nitrogen atmosphere, to a solution having 2.12
g (53.0 mmol) of 60% sodium hydride suspended in 80 ml of
DMF at 0°C, 7.05 g (44.1 mmol) of 2,2,4,4-tetramethyl-
20 1,3-pentanediol (compound (2)) dissolved in 15 ml of DMF,
was dropwise added over a period of 30 minutes, followed
by further stirring for 30 minutes. To this solution,
9.07 g (57.9 mmol) of 3-methoxybenzyl chloride (compound
(1)) dissolved in 15 ml of DMF was dropwise added over a
25 period of 30 minutes, followed by stirring for 12 hours.

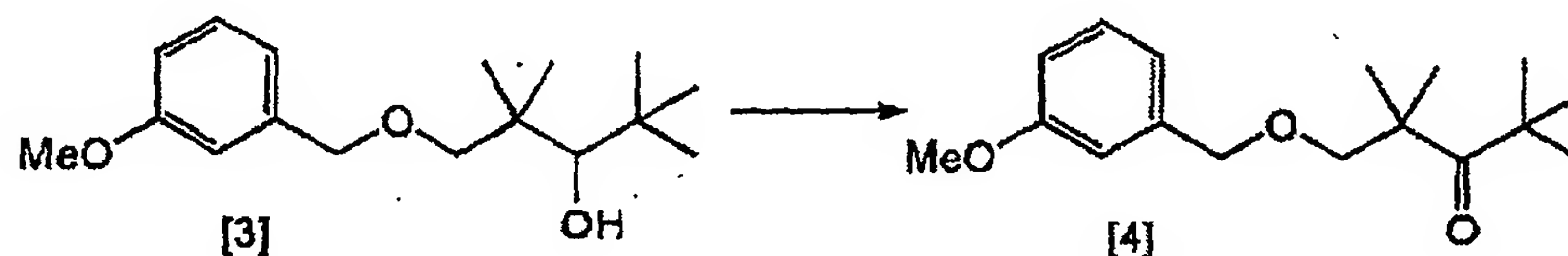
The reaction mixture was put into an aqueous saturated ammonium chloride solution and extracted with ethyl acetate. The extract layer was washed with a saturated sodium chloride aqueous solution, dried over anhydrous magnesium sulfate and then concentrated. The concentrated product was subjected to a silica gel column and eluted with a 10:1 mixed solvent of hexane and ethyl acetate, to obtain 10.7 g of 1-(3-methoxybenzyloxy)-2,2,4,4-tetramethyl-3-pentanol (compound (3)) in a yield of 86.7% as a colorless oily substance.

$^1\text{H NMR}$ (400 MHz, CDCl_3) ; δ 1.03 (s, 9H), 1.04 (s, 3H), 1.07 (s, 3H), 3.23 (d, $J=4.9\text{ Hz}$, 1H), 3.25 (d, $J=8.8\text{ Hz}$, 1H), 3.41 (d, $J=8.8\text{ Hz}$, 1H), 3.43 (d, $J=4.9\text{ Hz}$, 1H), 3.81 (s, 3H), 4.48 (s, 2H), 6.81-6.91 (m, 3H), 7.23-7.28 (m, 1H) ppm

IR (liquid film) ; 3502, 2954, 2870, 1489, 1457, 1267, 1080, 1053 cm^{-1}

Mass (m/z , %) ; 280 (M^+ , 2), 135 (31), 121 (100), 107 (8), 91 (9), 69 (13), 55 (14).

REFERENCE EXAMPLE 2



In a nitrogen atmosphere, 9.9 g of celite and 4.61 g (16.5 mmol) of 1-(3-methoxybenzyloxy)-2,2,4,4-tetramethyl-3-pentanol (compound (3)) were added to 75 ml

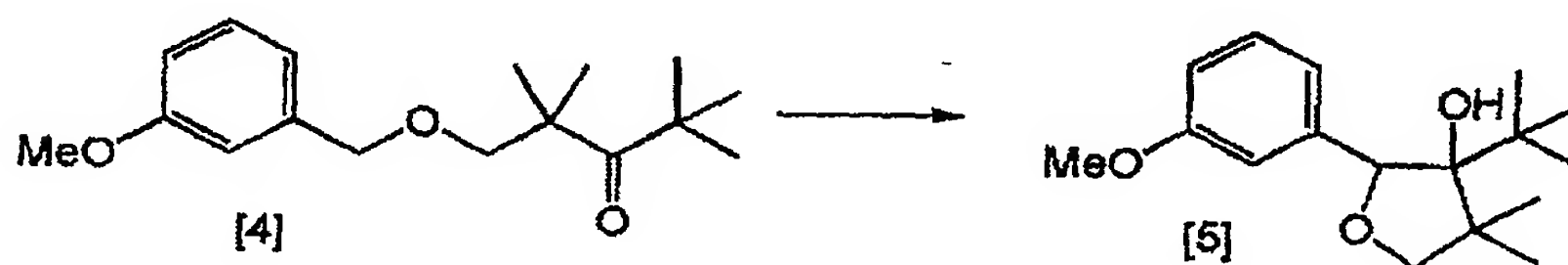
of dichloromethane at room temperature, followed by stirring. To this solution, 4.26 g (19.7 mmol) of PCC was added, followed by stirring for 7 hours. Then, 800 mg (3.71 mmol) of PCC was further added, followed by stirring overnight. To the reaction mixture, diethyl ether was added, followed by filtration with celite. The filtrate was concentrated, subjected to a silica gel column and eluted with a 10:1 mixed solvent of hexane and ethyl acetate, to obtain 4.32 g of 1-(3-methoxybenzyloxy)-2,2,4,4-tetramethyl-3-pentanone (compound (4)) in a yield of 94.4% as a colorless oily substance.

$^1\text{H NMR}$ (400 MHz, CDCl_3) ; δ 1.23 (s, 9H), 1.28 (s, 6H), 3.50 (s, 2H), 3.80 (s, 3H), 4.47 (s, 2H), 6.78–6.88 (m, 3H), 7.23 (t, $J=8.1\text{ Hz}$, 1H) ppm

IR (liquid film) ; 2959, 2870, 1658, 1480, 1466, 1458, 1267, 1108, 1049 cm^{-1}

Mass (m/z , %) ; 278 (M^+ , 100), 222 (50), 121 (31), 97 (5), 55 (8)

REFERENCE EXAMPLE 3



15

In a nitrogen atmosphere, 1.50 ml (11.4 mmol) of diisopropylamine and 6.60 ml (10.6 mmol) of a 1.6 M butyl

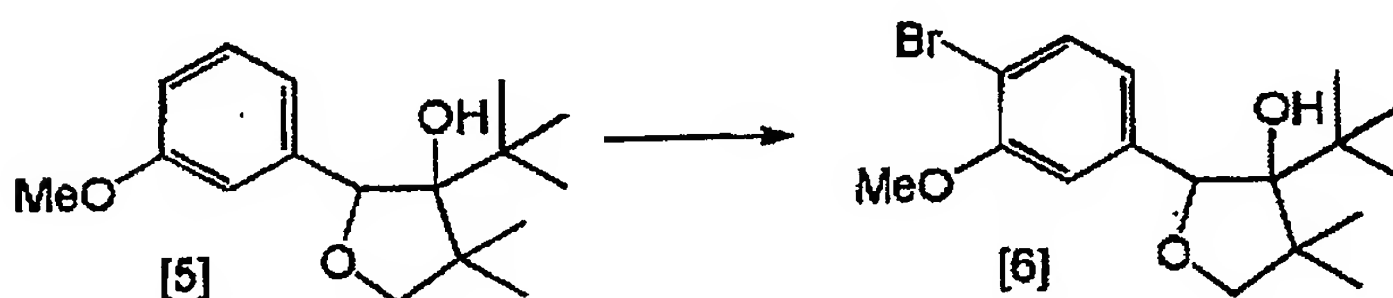
lithium hexane solution, were added to 15 ml of anhydrous THF at room temperature, followed by stirring for 30 minutes. To this solution, 1.48 g (5.32 mmol) of 1-(3-methoxybenzyloxy)-2,2,4,4-tetramethyl-3-pentanone (compound (4)) dissolved in 10 ml of THF, was added at -78°C, followed by stirring for 2 hours. The reaction solution was gradually heated to room temperature and stirred for 3 hours and 20 minutes. The reaction mixture was put into a saturated sodium chloride aqueous solution and extracted with ethyl acetate. The extract layer was washed with a saturated sodium chloride aqueous solution, dried over anhydrous magnesium sulfate and then concentrated. The concentrated product was subjected to a silica gel column and eluted with a 1:2 mixed solvent of hexane and ethyl acetate, to obtain 1.30 g of 3-t-butyl-3-hydroxy-2-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (compound (5)) in a yield of 87.8%. Melting point: 83.0-83.5°C (colorless granular crystals, recrystallized from hexane and ethyl acetate)

$^1\text{H NMR}$ (400 MHz, CDCl_3) ; δ 0.90 (broad s, 9H), 1.19 (s, 3H), 1.39 (s, 3H), 1.92 (s, 1H), 3.80 (q_{AB}, $J=8.1\text{ Hz}$, 2H), 3.80 (s, 3H), 5.00 (s, 1H), 6.80 (dd, $J=7.8$ and 2.4 Hz , 1H), 7.10 (d, $J=2.4\text{ Hz}$, 1H), 7.11 (d, $J=7.8\text{ Hz}$, 1H), 7.21 (t, $J=7.8\text{ Hz}$, 1H) ppm

IR (liquid film) ; 3493, 2962, 2881, 1591, 1481, 1278, 1070, 1048 cm^{-1}

Mass (m/z, %) ; 278 (M⁺, 1) , 260 (29) , 245 (100) , 203 (12) , 189 (45) , 135 (52) , 121 (10) , 107 (11) , 77 (9) , 55 (33) .

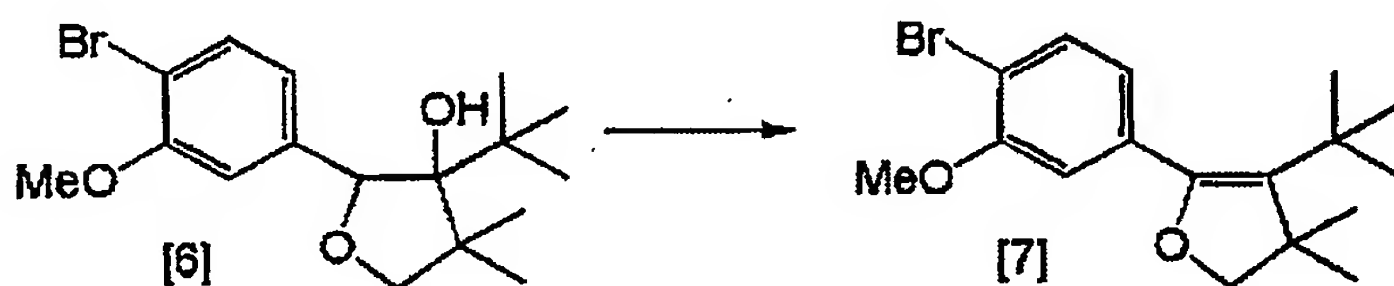
REFERENCE EXAMPLE 4



2.16 g (7.77 mmol) of 3-t-butyl-3-hydroxy-2-(3-methoxyphenyl)-4,4-dimethyl-2,3,4,5-tetrahydrofuran (compound (5)) was added to a mixed solvent of 20 ml of THF and 2 ml of H₂O, followed by stirring at 0°C. To this solution, 1.54 g (8.65 mmol) of NBS was added, and while gradually raising the temperature to room temperature, stirring was continued overnight. Then, 140 mg (0.787 mmol) of NBS was further added, followed by stirring for 6 hours. The reaction mixture was put into a saturated sodium chloride aqueous solution and extracted with ethyl acetate. The extract layer was washed sequentially with an aqueous sodium thiosulfate solution and a saturated sodium chloride aqueous solution, dried over anhydrous magnesium sulfate and then concentrated. The concentrated product was crystallized from a mixed solvent of ethyl acetate and hexane to obtain 1.323 g of 2-(4-bromo-3-methoxyphenyl)-3-t-butyl-3-hydroxy-4,4-dimethyl-2,3,4,5-tetrahydrofuran (compound (6)) in a yield of 47.7%.

^1H NMR (400 MHz, CDCl_3) ; δ 0.89 (s, 9H), 1.20 (s, 3H), 1.38 (s, 3H), 1.92 (s, 1H), 3.80 (q_{AB}, $J=8.3$ Hz, 2H), 3.89 (s, 3H), 4.98 (s, 1H), 7.02 (dd, $J=8.1$ and 2.0 Hz, 1H), 7.12 (d, $J=2.0$ Hz, 1H), 7.45 (d, $J=8.1$ Hz, 1H) ppm
Mass (m/z, %) ; 358 (M^++2 , 2.4), 356 (M^+ , 2.5), 340 (19), 338 (20), 325 (79), 323 (84), 215 (73), 213 (67), 201 (18), 199 (19), 109 (10), 55 (100).

REFERENCE EXAMPLE 5



5 4.68 g (13 mmol) of 4-t-butyl-5-(4-bromo-3-methoxyphenyl)-4-hydroxy-3,3-dimethyl-2,3,4,5-tetrahydrofuran (compound (6)) was added to 30 ml of anhydrous toluene at room temperature in a nitrogen atmosphere, followed by stirring for 10 minutes. To this
10 reaction solution, 0.27 g (1.4 mmol, 0.1 equivalent) of p-toluenesulfonic acid monohydrate was added, followed by stirring at 120°C for 30 minutes. The reaction solution was returned to room temperature, and this solution was put into a mixed solution of ethyl acetate and a
15 saturated sodium chloride aqueous solution to carry out extraction. The obtained organic layer was washed with a saturated sodium chloride aqueous solution. This organic

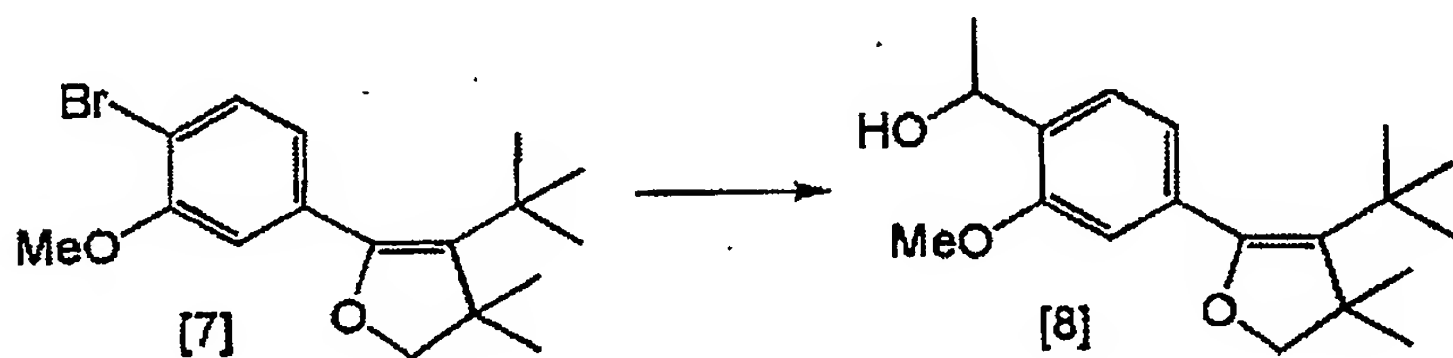
layer was dried over anhydrous magnesium sulfate and concentrated. The concentrated product was subjected to a silica gel column and eluted with a 2:1 mixed solvent of hexane and ethyl acetate to obtain 3.78 g (11.2 mmol) of 4-t-butyl-5-(4-bromo-3-methoxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (compound (7)) in a yield of 85% as a colorless oily substance.

^1H NMR (400MHz, CDCl_3) ; δ 1.06 (s, 9H), 1.33 (s, 6H), 3.87 (s, 2H), 3.9 (s, 3H), 6.79 (dd, $J=7.9$ and 1.6Hz, 1H), 6.82 (d, $J=1.6$ Hz, 1H), 7.49 (d, $J=7.9$ Hz, 1H) ppm

IR (liquid film) ; 2957, 2866, 1739, 1650, 1570, 1480, 1392, 1237, 1049, 1025, 795 cm^{-1}

Mass (m/z , %) ; 340 (M^++2 , 26), 338 (M^+ , 26), 325 (97), 323 (100), 283 (6), 282 (3), 281 (4), 187 (7), 185 (5), 172 (4), 170 (3), 77 (7), 55 (67).

REFERENCE EXAMPLE 6



10

To a solution having 5.05 g (14.88 mmol) of 4-t-butyl-5-(4-bromo-3-methoxyphenyl)-3,3-dimethyl-2,3-dihydrofuran (compound (7)) dissolved in THF (50 ml) at room temperature in a nitrogen atmosphere, a 1.63 M butyl lithium hexane solution (10.5 ml, 17.1 mmol) was added at -78°C, followed by stirring for 15 minutes. Then,

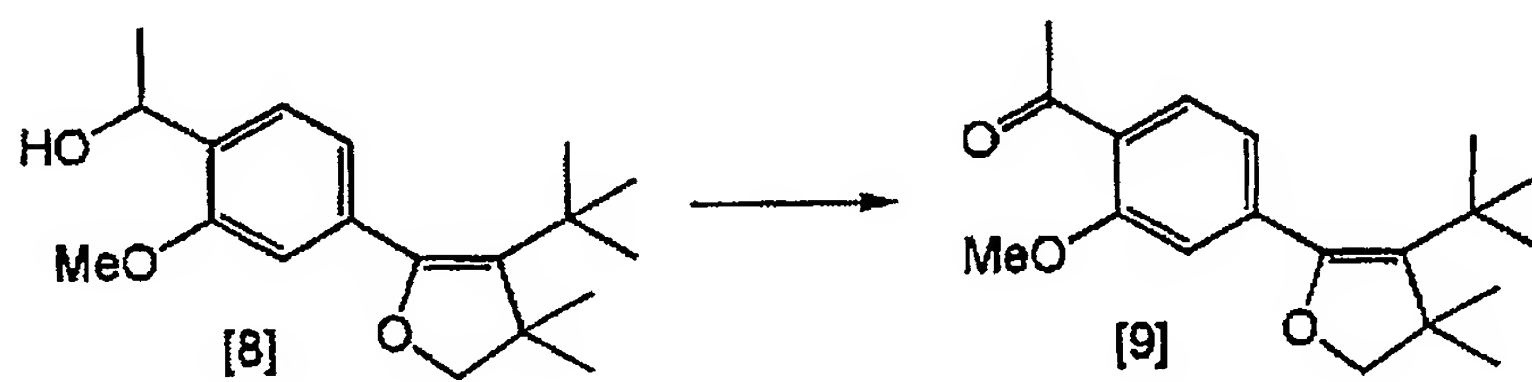
15

acetaldehyde (14.0 ml, 45.1 mmol) dissolved in hexane,
was added thereto, followed by stirring for 30 minutes.
To this reaction solution, a small amount of H₂O was
dropwise added to terminate the reaction, and the
5 reaction solution was put into a saturated ammonium
chloride aqueous solution (100 ml) and extracted with
ethyl acetate (100 ml). The aqueous layer was extracted
again with ethyl acetate (100 ml), and the extract was
put together with the previous organic layer, followed by
10 washing with a saturated sodium chloride aqueous solution
(200 ml × 3). The organic layer was dried over anhydrous
magnesium sulfate and concentrated, and the residue was
obtained as a slightly yellow oily substance (4.93 g).
This residue was subjected to silica gel column and
15 eluted with a 4:1 mixed solvent of hexane and ethyl
acetate to obtain the desired 4-t-butyl-5-[4-(1-
hydroxyethyl)-3-methoxyphenyl]-3,3-dimethyl-2,3-
dihydrofuran (compound (8)) as a colorless solid (3.73 g,
12.25 mmol, 82.3%).

¹HNMR (400 MHz, CDCl₃) ; δ 1.06 (s, 9H), 1.34
(s, 6H), 1.49 (d, J=6.2 Hz, 3H), 2.60 (d, J=4
. 9 Hz, 1H), 3.87 (s, 3H), 3.87 (s, 2H), 5.08 (p
e n t, J=6.2 Hz, 1H), 6.79 (s, 1H), 6.90 (d, J
=7.6 Hz, 1H), 7.28 (d, J=7.6 Hz, 1H) ppm
20

^{13}C NMR (125 MHz, CDCl_3) ; δ 22. 9, 27. 4, 32. 4, 32. 5, 47. 1, 66. 3, 83. 0, 111. 9, 122. 5, 125. 5, 125. 6, 133. 3, 136. 2, 149. 8, 156. 0 ppm
IR (KBr) ; 3491, 2962, 2870, 1651, 1604, 1461, 1402, 1229, 1129, 1088, 859 cm^{-1}
Mass (m/z , %) ; 304 (M^+ , 5), 303 (9), 287 (19), 271 (100), 177 (14), 161 (69), 149 (10), 135 (11), 111 (23), 55 (88).

REFERENCE EXAMPLE 7



To a solution having 4-t-butyl-5-[4-(1-
5 hydroxyethyl)-3-methoxyphenyl]-3,3-dimethyl-2,3-
dihydrofuran (compound (8)) (1.02 g, 3.351 mmol)
dissolved in DMSO (10 ml) and THF (5 ml) at room
temperature in a nitrogen atmosphere, triethylamine (1.65
ml, 11.8 mmol) and a pyridine/sulfur trioxide complex
10 (1.60 g, 10.1 mmol) was added, followed by stirring for 1
hour. This reaction solution was put into a saturated
sodium chloride aqueous solution (50 ml) and extracted
with ethyl acetate (50 ml). The aqueous layer was
extracted again with ethyl acetate (50 ml), and the
15 extract was put together with the previous organic layer,
followed by washing with a saturated sodium chloride

aqueous solution (100 ml \times 3). The organic layer was dried over anhydrous magnesium sulfate and concentrated, and the residue was obtained as a slightly yellow oily substance (1.02 g). This residue was subjected to a silica gel column and eluted with a 4:1 mixed solvent of hexane and ethyl acetate to obtain the desired 5-(4-acetyl-3-methoxyphenyl)-4-*t*-butyl-3,3-dimethyl-2,3-dihydrofuran (compound (9)) as a colorless solid (943 mg, 3.118 mmol, 93.0%).

$^1\text{H NMR}$ (400 MHz, CDCl_3) ; δ 1.07 (s, 9H), 1.34 (s, 6H), 2.61 (s, 3H), 3.89 (s, 2H), 3.92 (s, 3H), 6.89 (d, $J=1.3\text{ Hz}$, 1H), 6.95 (dd, $J=7.8$ and 1.3 Hz , 1H), 7.70 (d, $J=7.8\text{ Hz}$, 1H) ppm

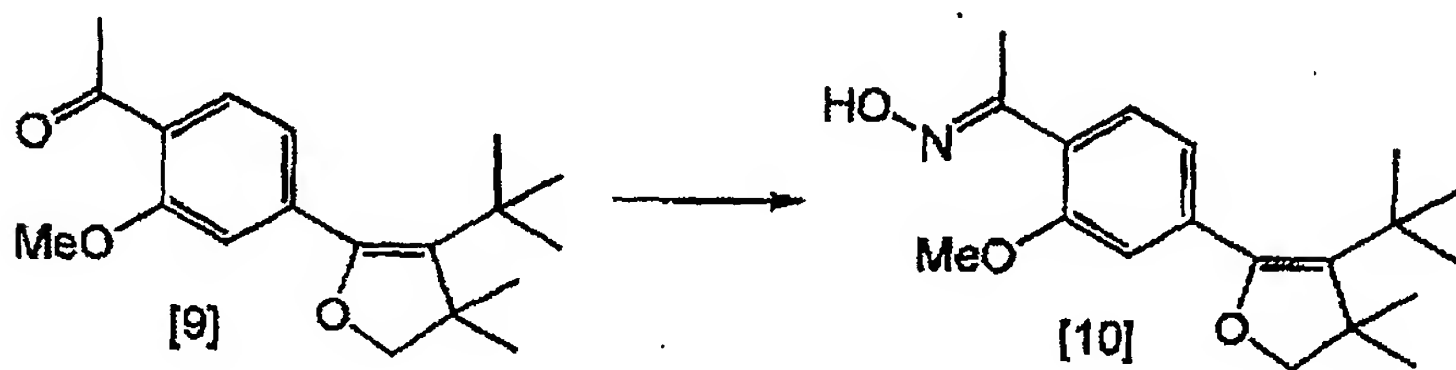
$^{13}\text{C NMR}$ (100 MHz, CDCl_3) ; δ 27.3, 31.8, 32.4, 32.4, 47.3, 55.5, 83.8, 113.1, 122.4, 126.

4, 127.7, 129.9, 141.8, 148.8, 158.4, 199.4 ppm

IR (KBr) ; 2957, 2868, 1676, 1600, 1560, 1463, 1401, 1232, 1053 cm^{-1}

Mass (m/z , %) ; 302 (M^+ , 27), 287 (100), 231 (40), 203 (14), 177 (78), 149 (9), 135 (6), 55 (48).

REFERENCE EXAMPLE 8



To a solution having 5-(4-acetyl-3-methoxyphenyl)-4-t-butyl-3,3-dimethyl-2,3-dihydrofuran (compound (9)) (1.35 g, 4.464 mmol) dissolved in ethanol (15 ml) at room temperature, sodium hydrogencarbonate (562 mg, 6.69 mmol) was added, and then hydroxylamine hydrochloride (472 mg, 6.79 mmol) was added, followed by refluxing at 90°C for 30 minutes. This reaction solution was put into a saturated sodium chloride aqueous solution (50 ml) and extracted with ethyl acetate (50 ml). The aqueous layer was extracted again with ethyl acetate (50 ml), and the extract was put together with the previous organic layer, followed by washing with a saturated sodium chloride aqueous solution (100 ml × 3). The organic layer was dried over anhydrous magnesium sulfate and concentrated, and the residue was obtained as a slightly yellow solid (1.38 g). The residue was subjected to a silica gel column and eluted with a 4:1 mixed solvent of hexane and ethyl acetate to obtain the desired 4-t-butyl-5-[4-(1-hydroxyiminoethyl)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (10)) as a colorless solid (1.11 g, 3.497 mmol, 78.3 %).

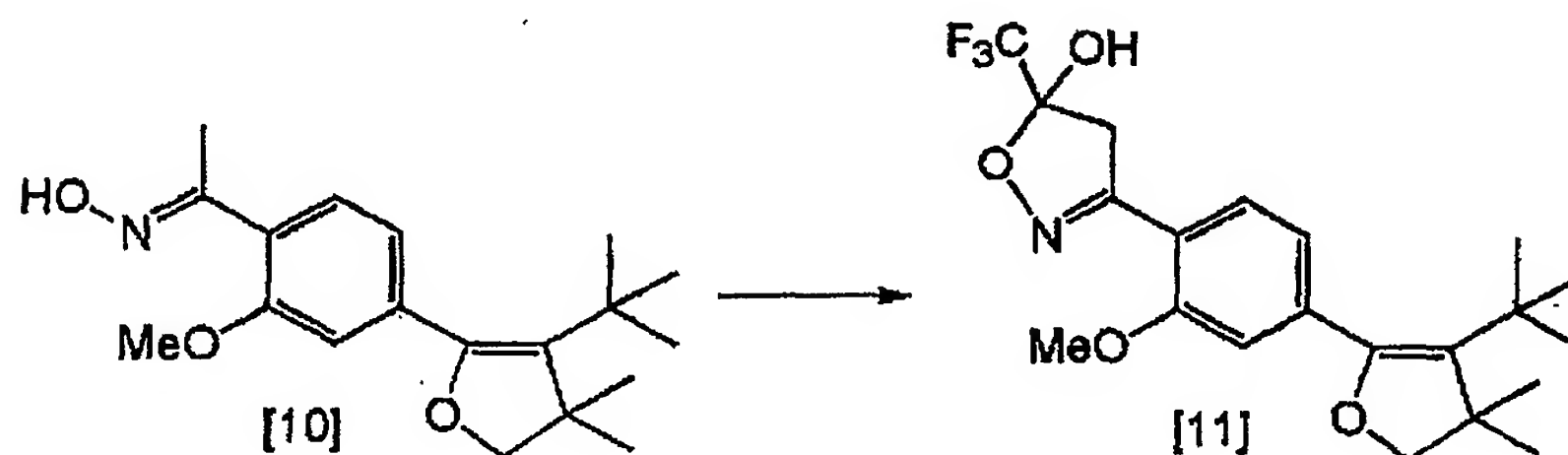
¹H NMR (400 MHz, CDCl₃) ; δ 1.07 (s, 9H), 1.34 (s, 6H), 2.22 (s, 3H), 3.84 (s, 3H), 3.88 (s, 2H), 6.83 (d, J=1.4 Hz, 1H), 6.90 (dd, J=7.6 and 1.4 Hz, 1H), 7.23-7.27 (m, 1H), 7.81 (br-s, 1H) ppm

^{13}C NMR (125 MHz, CDCl_3) ; δ 15. 1, 27. 4, 32. 4, 32. 5, 47. 2, 55. 5, 83. 1, 112. 6, 122. 3, 125. 9, 126. 4, 128. 8, 138. 1, 149. 5, 156. 5, 156. 9 ppm

IR (KBr) ; 3228, 2963, 2865, 1602, 1561, 1396, 1311, 1226, 1051, 930 cm^{-1}

Mass (m/z , %) ; 317 (M^+ , 29), 302 (100), 286 (32), 270 (13), 260 (10), 246 (18), 230 (11), 214 (14), 192 (14), 176 (7), 57 (4).

REFERENCE EXAMPLE 9



To a solution having 4-*t*-butyl-5-[4-(1-hydroxyiminoethyl)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (10)) (980 mg, 3.087 mmol) dissolved in THF (10 ml) at room temperature in a nitrogen atmosphere, 1.61 M butyl lithium hexane solution (4.20 ml, 6.76 mmol) was added at -78°C , followed by stirring for 5 minutes. Trifluoroacetic acid *S*-ethyl ester (0.50 ml, 8.90 mmol) was added thereto, and the mixture was gradually returned to room temperature and stirred for 1 day. This reaction solution was put into a saturated ammonium chloride aqueous solution (50 ml) and extracted with ethyl acetate (50 ml). The aqueous layer

was extracted again with ethyl acetate (50 ml), and the extract was put together with the previous organic layer, followed by washing with a saturated sodium chloride aqueous solution (100 ml \times 3). The organic layer was
5 dried over anhydrous magnesium sulfate and concentrated, and the residue was obtained as a slightly yellow oily substance (1.25 mg). This residue was subjected to a silica gel column and eluted with a 4:1 mixed solvent of hexane and ethyl acetate to obtain the desired 4-t-butyl-
10 5-[4-(5-trifluoromethyl-5-hydroxyisoxazolin-3-yl)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (11)) as a colorless solid (841 mg, 2.034 mmol, 65.9%).

^1H NMR (400 MHz, CDCl_3) ; δ 1.07 (s, 9H), 1.34 (s, 6H), 3.49 (br-s, 1H), 3.63 (d, $J=18.8$ Hz, 1H), 3.84 (d, $J=18.8$ Hz, 1H), 3.88 (s, 3H), 3.89 (s, 2H), 6.86 (d, $J=1.4$ Hz, 1H), 6.96 (d, $J=8.0$ and 1.4 Hz, 1H), 7.77 (d, $J=8.0$ Hz, 1H) ppm

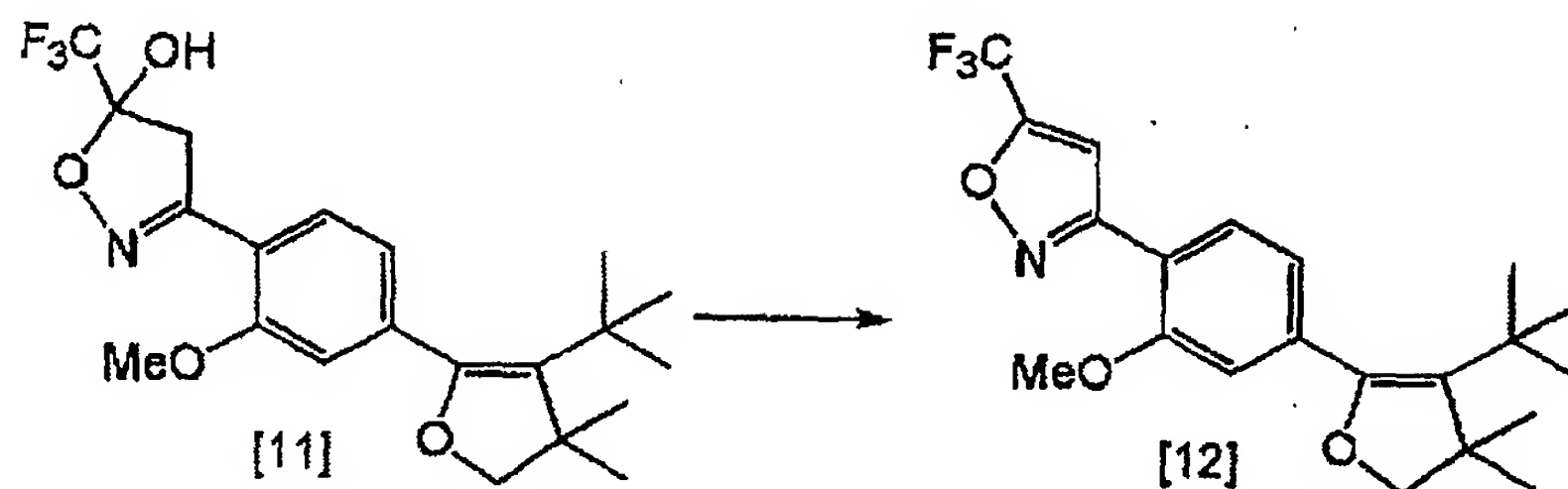
^{13}C NMR (100 MHz, CDCl_3) ; δ 27.3, 32.5, 32.5, 45.4, 47.3, 55.6, 83.1, 103.1 (q, $J=33.7$ Hz), 113.0, 116.4, 122.0 (d, $J=283.3$ Hz), 122.8, 126.7, 128.7, 140.1, 148.5, 155.7, 157.0 ppm

IR (KBr) ; 3329, 2962, 2873, 1605, 1466, 1410, 1185, 1050, 1005, 860 cm^{-1}

Mass (m/z , %) ; 413 (M^+ , 29), 398 (100), 380 (28), 342 (26), 324 (12), 288 (35), 270 (60), 214 (22), 160 (22), 57 (8).

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REFERENCE EXAMPLE 10



To a solution having 4-t-butyl-5-[4-(5-trifluoromethyl-5-hydroxyisoxazolin-3-yl)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (11)) (922 mg, 2.230 mmol) dissolved in toluene (10 ml) at room temperature, p-toluenesulfonic acid monohydrate (45.8 mg, 0.241 mmol) was added, followed by refluxing at 130°C for 1 hour. This reaction solution was put into a saturated sodium hydrogencarbonate solution (50 ml) and extracted with ethyl acetate (50 ml). The aqueous layer was extracted again with ethyl acetate (50 ml), and the extract was put together with the previous organic layer, followed by washing with a saturated sodium chloride aqueous solution (100 ml × 3). The organic layer was dried over anhydrous magnesium sulfate and concentrated, and the residue was obtained as a slightly yellow solid (930 mg). This residue was subjected to a silica gel column and eluted with a 4:1 mixed solvent of hexane and ethyl acetate to obtain the desired 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (12)) as a colorless

solid (748 mg, 1.983 mmol, 88.9%).

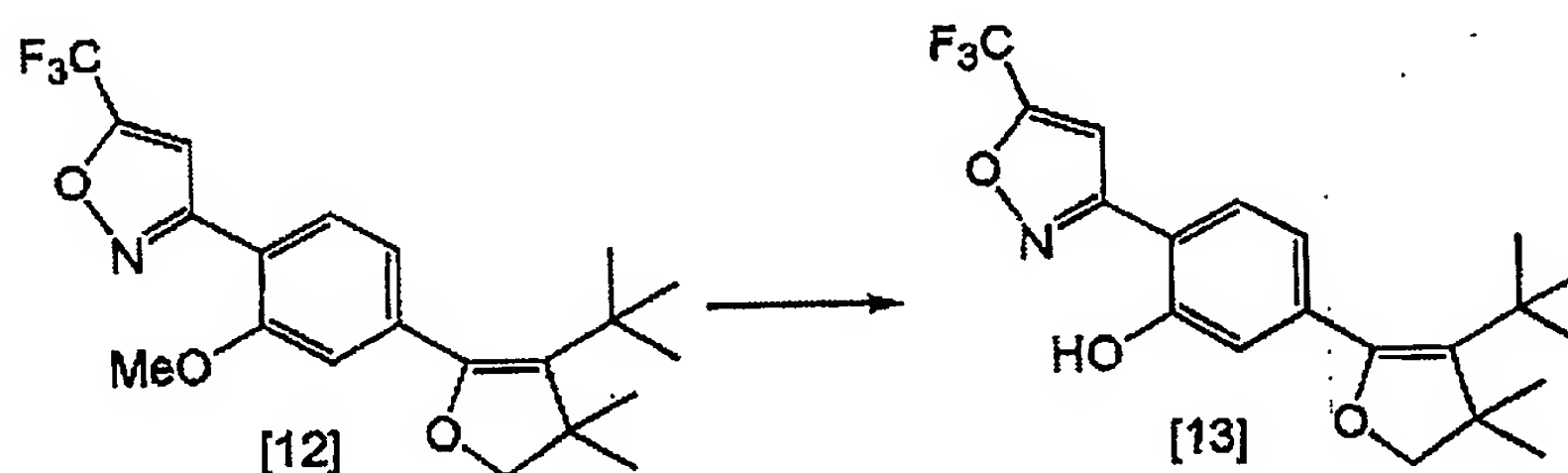
^1H NMR (400 MHz, CDCl_3) ; δ 1.09 (s, 9H), 1.36 (s, 6H), 3.90 (s, 2H), 3.93 (s, 3H), 6.95 (d, $J=1.2$ Hz, 1H), 7.02 (dd, $J=7.8$ and 1.2 Hz, 1H), 7.23 (d, $J=0.7$ Hz, 1H), 7.89 (d, $J=7.8$ Hz, 1H) ppm

^{13}C NMR (100 MHz, CDCl_3) ; δ 27.4, 32.5, 32.5, 47.3, 55.7, 83.6, 106.8 (d, $J=1.7$ Hz), 113.0, 115.8, 118.0 (d, $J=269.8$ Hz), 122.9, 126.4, 128.8, 140.2, 148.8, 156.6, 157.8 (q, $J=42.0$ Hz), 159.8 ppm

IR (KBr) ; 2961, 2870, 1606, 1450, 1313, 1178, 1152, 1052, 967, 834 cm^{-1}

Mass (m/z , %) ; 395 (M^+ , 22), 380 (100), 345 (16), 338 (19), 324 (25), 270 (53), 244 (10), 228 (7), 214 (9), 160 (13), 149 (10), 57 (15).

REFERENCE EXAMPLE 11



5

Ethanethiol (0.40 ml, 5.40 mmol) was added to DMF (3 ml) having 135 mg (3.38 mmol) of 60% sodium hydride suspended at 0°C in a nitrogen atmosphere, followed by stirring for 15 minutes. To this reaction solution, a solution having 4-t-butyl-5-[4-(5-trifluoromethyl-3-

10

isoxazoly1)-3-methoxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (12)) (664 mg, 1.679 mmol) dissolved in DMF (3 ml), was dropwise added, followed by heating at 140°C for 1 hours. This reaction solution was put into a saturated ammonium chloride aqueous solution (50 ml) and extracted with ethyl acetate (50 ml). The aqueous layer was extracted again with ethyl acetate, and the extract was put together with the previous organic layer, followed by washing with a saturated sodium chloride aqueous solution (100 ml × 3). The organic layer was dried over anhydrous magnesium sulfate and concentrated, and the residue was obtained as a slightly yellow solid (671 mg). This residue was subjected to a silica gel column and eluted with a 4:1 mixed solvent of hexane and ethyl acetate to obtain the desired 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazoly1)-3-hydroxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (13)) as a colorless solid (533 mg, 1.398 mmol, 83.3%).

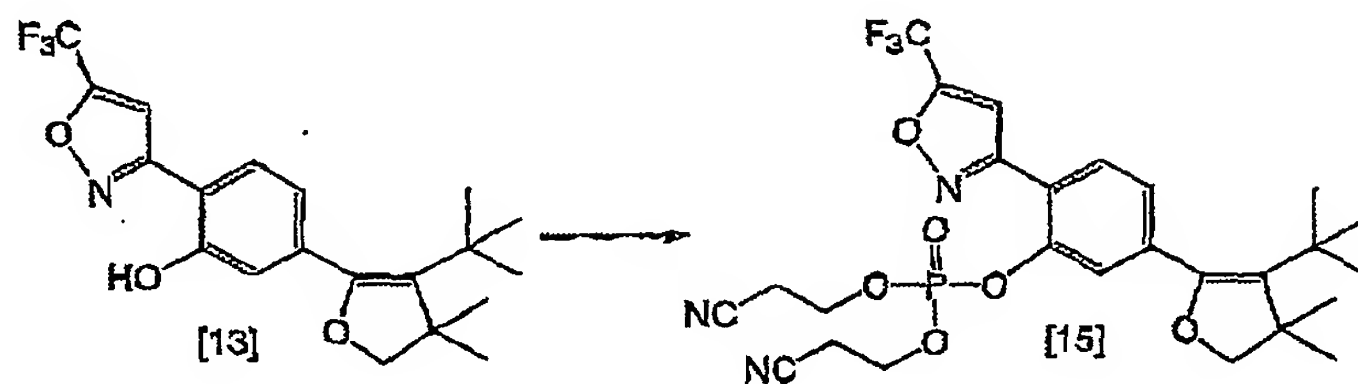
¹HNMR (400MHz, CDCl₃) ; δ 1.08 (s, 9H), 1.34 (s, 6H), 3.89 (s, 2H), 6.95 (dd, J=8.1 and 1.5 Hz, 1H), 7.06 (d, J=1.2 Hz, 1H), 7.57 (d, J=1.5 Hz, 1H), 7.84 (d, J=8.1 Hz, 1H), 10.6 (s, 1H) ppm

^{13}C NMR (100 MHz, CDCl_3) ; δ 27. 3, 32. 5, 32. 5, 47. 4, 83. 3, 109. 2, 118. 7 (q, $J=266.8\text{ Hz}$) , 119. 1, 121. 6, 126. 0, 127. 5 (d, $J=3.3\text{ Hz}$) , 138. 4 (q, $J=44.5\text{ Hz}$) , 142. 1, 148. 4, 157. 3, 163. 0 ppm

IR (KBr) ; 3355, 3148, 2960, 2868, 1629, 1576, 1494, 1330, 1179, 1147, 1052, 765 cm^{-1}

Mass (m/z , %) ; 381 (M^+ , 61) , 366 (100) , 324 (6) , 310 (88) , 278 (11) , 256 (85) , 228 (18) , 200 (17) , 57 (19) .

REFERENCE EXAMPLE 12

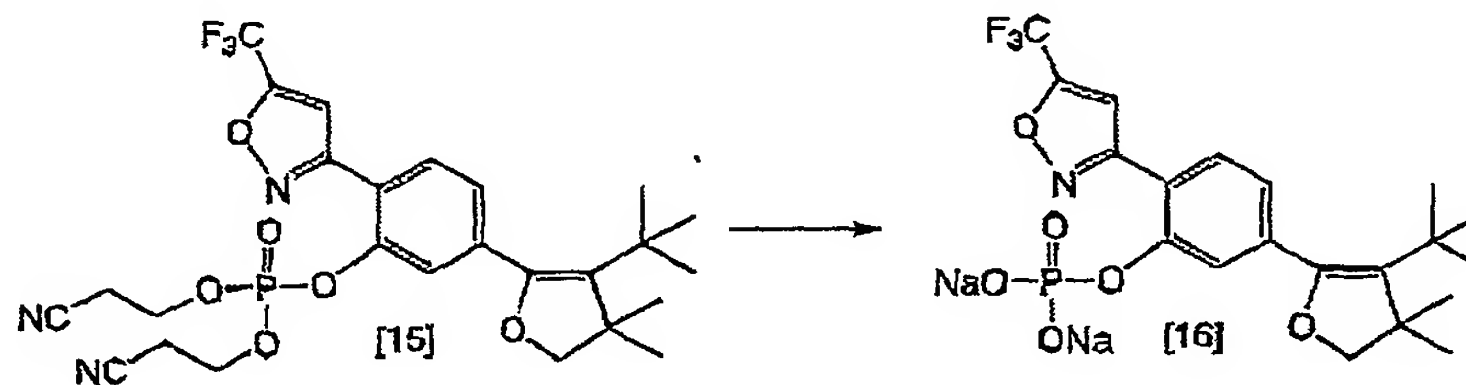


5 In a nitrogen atmosphere, 1.84 ml (22.8 mmol) of pyridine was added to 30 ml of dichloromethane at 0°C, and further, 1.33 ml (14.3 mmol) of phosphorus oxychloride was added, followed by stirring for 15 minutes. To this reaction solution, a solution having 4-
10 t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound 13)) (1.44 g, 3.78 mmol) dissolved in dichloromethane (12 ml), was dropwise added, followed by stirring at 0°C for 2 hours. Further, the reaction solution was gradually
15 returned to room temperature and stirred for 1 day. The reaction solution was again cooled to 0°C, and 3.68 ml

(45.5 mmol) of pyridine was added. Further, 3.20 ml
(47.3 mmol) of ethylene cyanohydrin was added, and the
mixture was gradually returned to room temperature and
stirred for 1 day. The reaction solution was put into
5 pure water (50 ml) and extracted with ethyl acetate (50
ml). The aqueous layer was again extracted with ethyl
acetate (50 ml), and the extract was put together with
the previous organic layer, followed by washing with pure
water (100 ml \times 3). The organic layer was dried over
10 anhydrous magnesium sulfate and concentrated to obtain
the desired phosphoric acid 5-(3-t-butyl-4,4-dimethyl-
4,5-dihydrofuran-2-yl)-2-(5-trifluoromethylisoxazol-3-
yl)phenylester bis-(2-cyanoethyl)ester (compound (15)) as
a slightly yellow oily substance (2.10 g, 3.70 mmol,
15 98.1%).

^1H NMR (500MHz, CDCl_3); δ 1.08 (s, 9H), 1.35 (s, 6H),
2.80 (m, 4H), 3.90 (s, 2H), 4.45 (m, 4H), 7.33 (dd, 1
H), 7.53 (d, 1H), 7.63 (d, 1H), 8.09 (dd, 1H), ppm

REFERENCE EXAMPLE 13

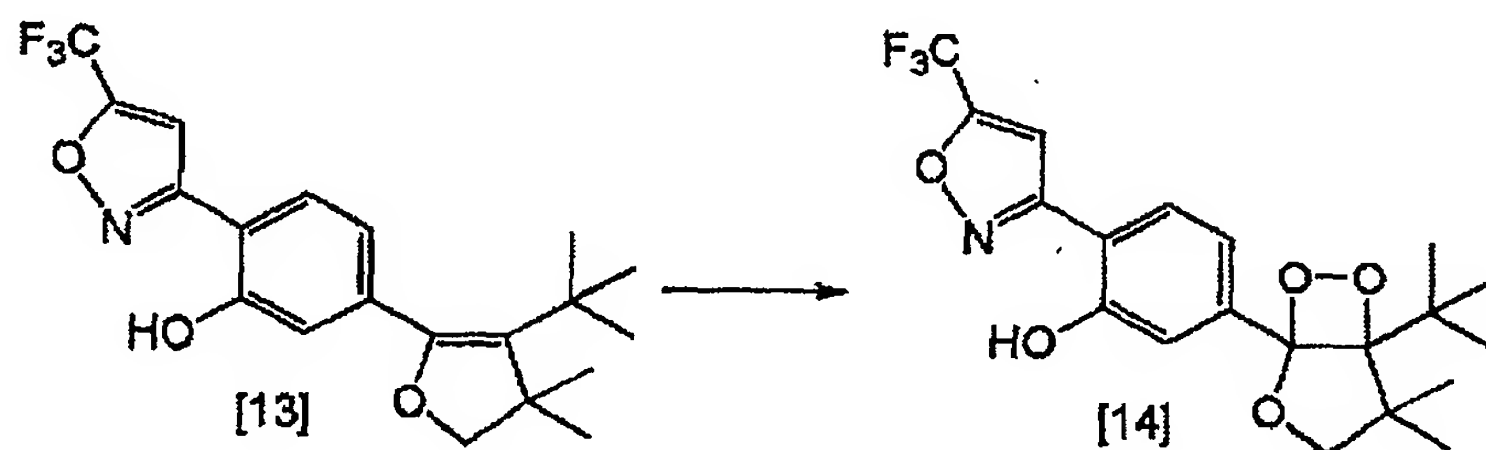


20 At room temperature, a 28% sodium methylate methanol
solution (1.6 ml) was added to a solution having
phosphoric acid-5-(3-t-butyl-4,4-dimethyl-4,5-

2040ED 44T5001
dihydrofuran-2-yl)-2-(5-trifluoromethylisoxazol-3-yl)phenylester bis-(2-cyanoethyl)ester (compound (15)) (1.20 g, 2.11 mmol) dissolved in methanol (40 ml), followed by stirring for 1 hour and 30 minutes. To this reaction solution, a saturated sodium hydrogencarbonate aqueous solution (2.0 ml) was added and further stirred for 30 minutes and then concentrated to obtain a white solid. To this solid, methanol (20 ml) was added, and insolubles were removed by filtration. The filtrate was concentrated to obtain the desired phosphoric acid mono-[5-(3-t-butyl-4,4-dimethyl-4,5-dihydrofuran-2-yl)-2-(5-trifluoromethylisoxazol-3-yl)phenyl]ester disodium salt (compound (16)) as a white solid (0.69 g, 1.37 mmol, 64.5%).

¹HNMR (500 MHz, CD₃OD); δ 1.11 (s, 9H), 1.34 (s, 6H), 3.83 (s, 2H), 6.98 (dd, 1H), 7.78 (d, 1H), 7.85 (d, 1H), 8.05 (d, 1H), ppm

EXAMPLE 1



To a solution having 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-3,3-dimethyl-2,3-dihydrofuran (compound (13)) (80.0 mg,

0.2098 mmol) dissolved in CH₂Cl₂ (5 ml) at 0°C in an oxygen atmosphere, TPP (2.1 mg) was added, and then irradiation by a sodium lamp was carried out, followed by stirring for 30 minutes. This reaction solution was concentrated, and the residue was obtained as a green solid (81.2 mg). This residue was subjected to a silica gel column and eluted with a 20:1 mixed solvent of hexane and diethyl ether to obtain the desired 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (compound (14)) as a slightly yellow solid (75.3 mg, 0.1822 mmol, 86.8%).

¹H NMR (400 MHz, CDCl₃) ; δ 1.02 (s, 9H), 1.17 (s, 3H), 1.39 (s, 3H), 3.85 (d, J=8.3 Hz, 1H), 4.60 (d, J=8.3 Hz, 1H), 7.28 (dd, J=8.3 and 1.5 Hz, 1H), 7.39 (d, J=1.5 Hz, 1H), 7.59 (d, J=1.5 Hz, 1H), 7.91 (d, J=8.3 Hz, 1H), 10.67 (s, 1H) ppm

¹³C NMR (100 MHz, CDCl₃) ; δ 18.5, 25.1, 27.0, 36.8, 45.7, 80.5, 105.4, 110.3, 115.9, 117.9, 118.7 (q, J=267.0 Hz), 119.7, 126.0, 127.5 (d, J=2.5 Hz), 138.7 (q, J=44.5 Hz), 141

. 8, 157.3, 162.7 ppm

IR (KBr) ; 3144, 2975, 2898, 1613, 1550, 1494, 1371, 1331, 1219, 1149, 1035, 959, 872 cm⁻¹

Mass (m/z, %) ; 413 (M⁺, 1), 381 (13), 366 (20), 357 (28), 328 (7), 273 (33), 256 (100), 228 (13), 200 (14), 57 (25)

EXAMPLE 2

1 ml of a 1.00×10⁻⁵M acetonitrile solution of 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (compound (14)) obtained in Example 1, was added at 40°C to 2 ml of a 1.00×10⁻²M DMSO solution of tetrabutylammonium fluoride. The luminescence at that time was measured by a fluorescence analyzer. The luminous quantum yield at that time was estimated to be 0.44, the half value period of luminescence was 1,400 seconds, and λ_{max} was 481 nm.

EXAMPLE 3

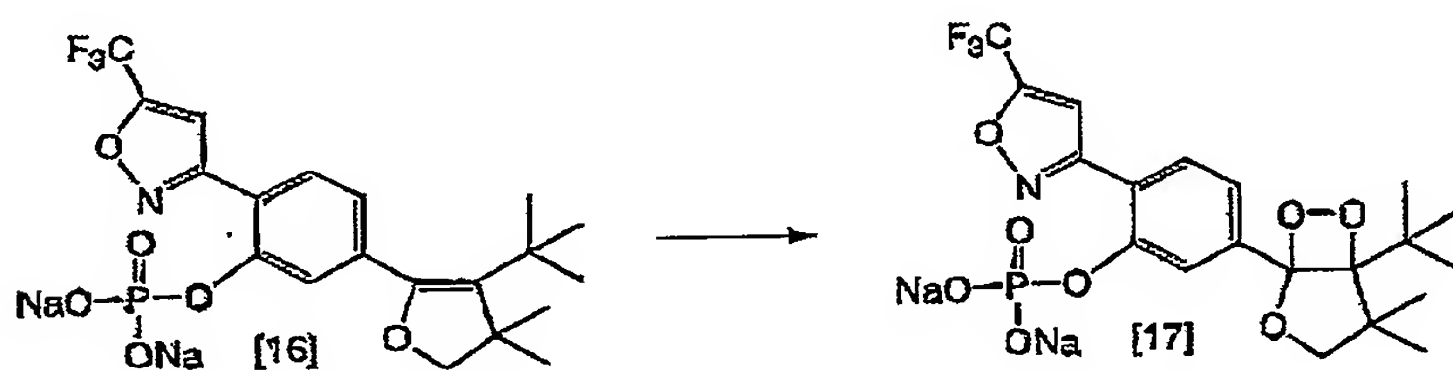
1 ml of a 1.00×10⁻⁴M acetonitrile solution of 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (compound (14)) obtained in Example 1, was added at 40°C to 2 ml of a 0.1N solution of sodium hydroxide. The luminescence at that time was measured by a fluorescent analyzer. The luminous quantum yield at that time was estimated to be 0.39, the half

value period of luminescence was 2,700 seconds, and λ_{\max} was 479 nm.

EXAMPLE 4

0.1 ml of a 1.00×10^{-3} M acetonitrile solution of 4-t-butyl-5-[4-(5-trifluoromethyl-3-isoxazolyl)-3-hydroxyphenyl]-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane (compound (14)) obtained in Example 1, was added at 40°C to 2 ml of a 0.1N solution of sodium hydroxide + 0.9 ml of distilled water. The luminescence at that time was measured by a fluorescent analyzer. The luminous quantum yield at that time was estimated to be 0.24, the half value period of luminescence was 1,200 seconds, and λ_{\max} was 476 nm.

EXAMPLE 5



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In an oxygen atmosphere at 0°C, TPP (2.0 mg) was added to a mixed solution having phosphoric acid mono-[5-(3-t-butyl-4,4-dimethyl-4,5-dihydrofuran-2-yl)-2-(5-trifluoromethylisoxazol-3-yl)phenyl]ester disodium salt (compound (16)) (65.0 mg, 0.129 mmol) dissolved in methanol (4 ml) and dichloromethane (15 ml), followed by stirring for 2 hours under irradiation by a sodium lamp. The reaction mixture was concentrated, and methanol was added to the concentrate, whereupon insolubles were

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filtered off by means of a 0.45 μ m
polytetrafluoroethylene filter, followed by concentration
again. The concentrate was dissolved in pure water (1.5
ml) and subjected to HPLC employing a polymer type
5 reversed phase C18 fractionation column, and the fraction
eluted with water and acetonitrile was subjected to
freeze drying to obtain the desired phosphoric acid mono-
[5-(5-t-butyl-4,4-dimethyl-2,6,7-
trioxabicyclo[3.2.0]hept-1-yl)-2-(5-
10 trifluoromethylisoxazol-3-yl)phenyl]ester disodium salt
(compound (17)) as a white solid (52.0 mg, 0.097 mmol,
75.3%).

^1H NMR (500 MHz, CD_3OD); δ 1.04 (s, 9H), 1.14 (s, 3H),
1.44 (s, 3H), 3.83 (d, 1H), 4.49 (d, 1H), 7.33 (dd, 1
H), 7.79 (d, 1H), 7.91 (dd, 1H), 8.35 (d, 1H) ppm

The 1,2-dioxetane derivative (1) of the present
15 invention is capable of exhibiting stable luminescence
having a high quantum yield and is a stable compound
having high thermal stability such that depending upon
the cold storage, no decomposition product will be
observed upon expiration of one year. Accordingly,
20 measurement of the luminescence can be carried out simply
and efficiently, and thus, it is useful, for example, in
the field of clinical tests. Further, the 1,2-dioxetane
derivative (I) of the present invention not only has both
high thermal stability and high luminous efficiency, but
25 also makes it possible to omit an enhancer itself or an

operation to add an enhancer in a protic solvent, whereby costs and time can be saved.

The entire disclosure of Japanese Patent Application No. 2001-65347 filed on March 8, 2001 including
5 specification, claims and summary are incorporated herein by reference in its entirety

2001-65347